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ORIGINAL ARTICLE

Phyto – Monoterpene linalool as precursor to synthesis epoxides and hydroperoxides as anti carcinogenic agents via thermal and photo chemical oxidation reactions

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KEYWORDS

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Epoxidation;
Photosensitization;
Hydroperoxides;
Anticancer;
Antioxidant

Abstract Linalool (**I**) was extracted from *Zingiber officinale*. The oxidation of linalool using *m*-chloroperbenzoic acid at room temperature gave the mixture of 2,2,6-trimethyl-6-vinyl-tetrahy dro-pyran-3-ol (**IIa) &** 2-(5-methyl-5-vinyl- tetrahydro-furan-2-yl)-propan-2-ol (**IIa′**), which cannot be separated. Whereas, the photo-epoxidation of it using hydrogen peroxide gave the above mixture **IIa**/**IIa**′, beside 2,2,8-trimethyl-6-oxianyl-tetrahydro-pyran-3-ol **(IIb)**. It was evaluated for anti-oxidant, using erythrocyte hemolysis and ABTS methods. It showed inhibitory effect in case of erythrocyte hemolysis and low inhibitory effect in case of ABTS method. Photosensitization reactions of linalool with tetraphenyl porphyrin or chlorophyll produced a mixture of two isomeric hydroperoxides, 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol **(IIIa)** & 6-hydroperoxy-3,7-dimethyl octa-1,7-dien-3-ol **(IIIb),** which can be successfully separated**.** On the other hand, the cyto-toxic activity of linalool (**I**) was tested against epdermoid carcinoma (HEP2), it has medium effect. Whereas, in case of human prostate cancer (PC-3), it has weak effect. From this study concludes that ginger has good antioxidant potential and this spice can be used to produce novel natural antioxidants and flavoring agent like linalool which recommends it as an active therapy for humans. Monoterpene linalool abstract singlet oxygen ($^1O_2$) by photosensitization reactions to produce these hydroperoxides, which are caused relatively little oxidative DNA damage.

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1. Introduction

Ginger (***Zingiber officinale***) belongs to the family *Zingiberaceae* that has an subsurface stem (called a rhizome). Ginger rhizome is usually used as powder or paste. It has a prolonged history of being used as herbal medicine since ancient times (Altman

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and Marcussen, 2001). Ginger has been used in the treatment of arthritis, rheumatological conditions and muscular discomfort (Grant and Lutz, 2000). Ginger has antioxidant activity (El-ghorab et al., 2010) and can be used *in vitro* as anti ovarian cancer cells (Choudhury et al., 2010).

Essential oil of ginger contains sesquiterpenes, and a small monoterpenes as (Linalool, α-Terpineol, ---) (Harold, 2004).

The highly pleasant odor of acyclic monoterpene alcohols is linalool which plays an important part in the composition of perfumes and various other formulations. Since linalool owns an asymmetric carbon atom, it is capable of existing in optically active as a mixture of d and l forms, with one form predominating (Sköld et al., 2004; Bentley, 2006).

Oxidation of phyto-monoterpene considers an important method in the manufacture of many worthy compounds, which find interest in the industrial. The essential basis for much preparation of valuable organic compounds as diols, epoxy resins, is epoxide. The epoxidation of olefins in air can be carried out using some organic – inorganic catalysts with good selectivities and medium enantioselectivities (Cho et al., 2006, 2007; Corma et al., 2009). It is renowned that some monoterpenes undergo oxidation by the action of hydrogen peroxide under thermal conditions to give the corresponding epoxy derivatives O.I. (Yarovaya et al., 2002; Yarovaya et al., 2003). On the other hand, unsaturated terpenes are able of trapping activated oxygen species *in vivo* to give intermediate epoxides and hydroperoxides, which can alkylate or damage DNAs and proteins (Elgendy and Al-Ghamdy, 2007; Richter et al., 2003).

Recently, oxidation is enhanced by irradiation to form hydroperoxides, which can be used instead of oxygen as oxidizing agents (Elgendy, 2004). In addition, the oxidation in photooxidation reactions can be initiated by attack of reactive singlet oxygen, in the presence of oxygen, light energy and photosensitizers via ene mechanism to produce hydroperoxides (Elgendy, 1998). Hydroperoxides may lead to secondary oxidation products having various chemical functional groups (hydroxy, oxo, and epoxy derivatives) (Geraghty, 2005).

No attention has been paid to synthesis such epoxides via photochemical oxidation reactions. Taking into account therapeutic importance of monoterpene compounds, we believed it to be relevant to examine linalool (**I**) oxidation reactions to produce new epoxides and hydroperoxides as potential biologically active compounds, to discuss and prove its ability as antioxidant compound.

2. Materials and methods

2.1. Materials

Linalool (**I**) was isolated from essential oil of Ginger rhizome (***Zingiber officinale***). Perkin–Elmer 16 FPC FT-IR spectrophotometer was used to determine the IR spectra on a thin film (neat). Bruker Avance DPX 400 instrument (400 MHz for 1H) was used to measure the NMR spectra from solutions in $CDCl_3$. Joel JMS 600H mass spectrometer was used to determine gas chromatography–mass spectrometry. Photoinitiation reactions were carried out using a sodium lamp as irradiation source. Silica gel (Mecherey-Nagel) was used to perform thin-layer chromatography (TLC) and preparative thin-layer chromatography. Rotary evaporator at (20 °C, 15 mm) was used to remove solvents from reaction mixtures. Chemical reagents are in pure grade.

2.2. Methods

2.2.1. Photochemical epoxidation of linalool (*I*) with hydrogen peroxide:

Hydrogen peroxide 30% solution, was mixed drop wise to a solution of linalool (**I**) (5 mmol), according to the method of (Elgendy and Abou-Elzahab, 1998). The gummy material can be separated through column chromatography on silica gel with mixture of petroleum ether and ethyl acetate (9:1) as eluent to isolate 0.14 g of isomer mixture **IIa**/**IIa′** and 0.16 g of compound **IIb**, ratio **IIa**/**IIa′**: **IIb** = 52:48, overall yield 40% (Table 1).

2.2.2. Oxidation of linalool (*I*) with m-chloroperbenzoic acid

m-Chloroperbenzoic acid 80% solution was mixed to a solution of linalool (**I**) (5 mmol) at zero degree, according to (Elgendy, 2008) method. The gummy material can be separated through column chromatography on silica gel with mixture of petroleum ether and ethyl acetate (9:1) as eluent to isolate 0.6 g (80%) of isomer mixture **IIa**/**IIa′** as a viscous oil (Table 2).

2.2.3. Photosensitized oxygenation of linalool (*I*)

A continuous stream of dry oxygen being passed through a solution (0.01 mol) of compound **I** in chloroform or ethanol containing the corresponding singlet oxygen sensitizer, according to the method of (Elgendy, 1998). The isomer mixture **IIIa**/**IIIa′** can be separated, with mixture of petroleum ether and ethyl acetate (7:3) as eluent through silica gel column chromatography. The reaction conditions (solvent, sensitizer, reaction time) and yields of the photoproducts are given in (Table 3).

Table 2 Oxidation of linalool (I) with *m*-chloroperoxybenzoic acid.

Comp no.	Starting Wt. gm	Yield gm	Epoxide Prod.
Linalool (I)	0.75	0.6	**IIa**/**IIa′**: 80%

Table 1 Photochemical epoxidation of natural terpene I with hydrogen peroxide.

Comp. No.	Start Wt Gm	Irrad. Time (h)	Yield gm %	Yield of product
Linalool (I)	0.75	51	0.30, 40%	**IIa**/**IIa′** = 52% **IIb** = 48%

Table 3 Photosensitized oxygenation of natural terpene I.

Comp no.	Starting Wt. gm	Sens.	Reaction time	Yield gm,%	Yield Prod. %
I	1.5 gm	TPP	14	0.7, 46%	**IIIa: 60%, IIIb:40%**
		Cl	16	0.4, 26.6%	**IIIa: 60%, IIIb:40%**

TPP = Tetraphenyl porphyrin Cl = Chlorophyll.

*2.2.4. Study on photoinduced DNA damage in the presence of hydroperoxides **IIIa** and **IIIb***

A mixture of two hydroperoxides **IIIa** and **IIIb** (1 mg) was mixed with 1 ml, DNA in saline solution. It can be determined the damaging effect by the gel electrophoresis technique according to the method of (Kochevar and Dumn, 1990). Hydroperoxides **IIIa** and **IIIb** induced a moderate degree of DNA damage after irradiation for 25 h, and high degree of DNA damage after irradiation for 45 h.

*2.2.5. Determination of antioxidant activity to linalool (**I**) with (ABTS) assay*

To linalool (I), 2 ml. of ABTS solution was mixed, according to (Lissi, 1999) method. The absorbance (A test) was measured. The inhibition percentage was determined through lowering in color sharpness and can be computed as the next:

$$\% \text{ Inhibition} = (\text{A control} - \text{A test})/\text{A control} \times 100$$

Vitamin C can be used as standard anti-oxidant (positive control) (Table 4).

2.2.6. Assay for erythrocyte hemolysis

From plasma, erythrocytes were isolated. A 10% suspension of erythrocytes was mixed with linalool (I) solution according to method of (Tomaino, 2005), to be tested at different concentrations. Hemolysis percentage was calculated by equation $(1 - A/B) \times 100\%$. The absorbance B can be determined at 540 nm.

L-Ascorbic was used as a positive control (Table 5).

2.2.7. In vitro cytotoxicity activity evaluation

The inhibitory effect of linalool (I) can be determined on epdermoid carcinoma (HEP2) and human prostate cancer (PC-3) cell lines growth using the MTT assay. According to method of Mosmann (1983), the percentage of relative cell viability can be determined and computed according to the following:

Table 4 Anti-oxidant activity screening of linalool (I) by ABTS method.

Method	Anti-Oxidant Assays	
	ABTS	
	Abs(control) − Abs(test)/Abs(control) × 100	
Compounds	Absorbance of samples	% inhibition
Control of ABTS	0.532	0
Ascorbic-acid	0.057	89.3%
Linalool (I)	0.415	22.0%

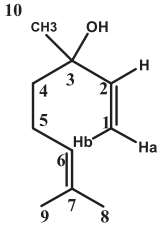
Table 5 Anti-oxidant activity screening of linalool (I) by Erythrocyte hemolysis method.

Compounds	Anti-hemolytic Assay	
	Erythrocyte hemolysis A/B × 100	
	Absorbance of samples (A)	% hemolysis
Absorbance of H_2O (B)	0.810	–
Vit - C	0.032	3.9%
Linalool (I)	0.058	7.2%

Relative cell viability % = Absorbance of treated cells/Absorbance of untreated cells × 100. IC50 value of **I** is its concentricity that cause death of 50% of the cells (Tables 6 and 7) and Figs. 1 and 2.

2.3. Spectral measurements

Linalool (I)



3,7-dimethylocta-1,6-dien-3-ol (I): Colorless oil, $C_{10}H_{18}O$ (*M* 154.250). IR spectrum, ν, **cm**$^{-1}$: 3464 (OH), 2967 (CH. Str.), 1641 (C═C) 1117 (C─O). ^{1}H NMR spectrum, δ, ppm: 1.28 s (3H, $^{10}CH_3$), 1.57 comp.pat. (2H, H-4), 1.6 s (3H, $^8CH_3$), 1.70 s (3H, $^9CH_3$), 2.0 comp.pat. (2H, H-5), 2.19 s (1H, OH), 5.06 d.d (1H, H-1^a, *J* = 7, 1 Hz), 5.13 comp. pat. (1H, H-6), 5. 21 d.d (1H, H-1^b, *J* = 11. 1 Hz), 5.91 d.d (1H, H-2, *J* = 11, 7 Hz). ^{13}C NMR spectrum, δC, ppm: 17.7(^{9}C),

Table 6 Cytotoxic activity of linalool (I) against epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) cell lines.

Compounds	In vitro cytotoxicity IC50[a]	
	HEP2	PC3
[b]DOX	6.32 ± 0.3	8.87 ± 0.6
L	25.23 ± 2.3	46.01 ± 3.4

[a] IC50: 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic).
[b] DOX: Doxorubicin.

Table 7 Relative viability % of epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) cell lines after treating by Linalool (I).

Relative viability of cells (%)		
Compounds	HEP2	PC3
DOX (μM)		
100	7.9	8.8
50	18.0	16.3
25	27.3	21.7
12.5	33.7	38.9
6.25	46.2	59.2
3.125	63.4	73.6
1.56	81.3	95.3
L (μg/ml)		
100	23.7	34.8
50	37.5	47.1
25	44.9	61.2
12.5	61.8	75.0
6.25	79.2	97.4
3.125	98.3	100
1.56	100	100

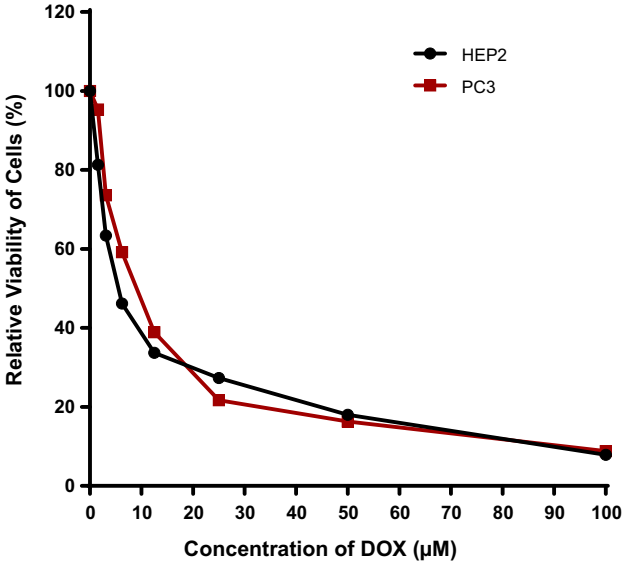


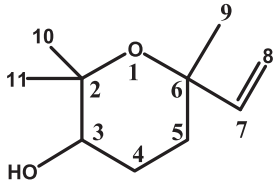
Fig. 1 Effect concentration of DOX on relative viability % epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) cell lines.

22.8 (^{5}C), 25.7 (^{8}C), 27.9 (^{10}C), 42.0 (^{4}C), 73.5 (^{3}C), 111.7 (^{1}C), 124.3(^{6}C), 132.0 (^{7}C), 145.0 (^{2}C). GC–MS data: retention time **12.859** min; *m/z* (*I*rel, %): 154 (2) $[M]^+$, 136 (15) $(M\text{-}H_2O)^+$, 121(25) $(M\text{-}CH_5O)^+$, 83 (20) $(C_6H_{11})^+$, 71(1 0 0) $(C_4H_7O)^+$, 55 (65) $(C_4H_7)^+$.

Linalool Thermal Epoxidation:

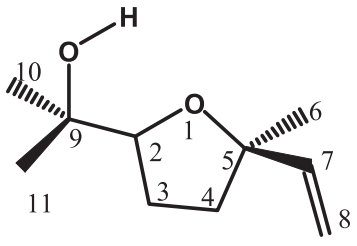
Mix of two compounds IIa/IIa′

The first Compound:



2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol (IIa): ^{1}H NMR spectrum, δ, ppm: 1.20 s (3H, $^{9}CH_3$), 1.31 s (3H, $^{11}CH_3$), 1.33 s (3H, $^{10}CH_3$), 1.70 comp. pat. (2H, H-5), 1.89 comp. pat. (2H, H-4), 2.9 d (1H, OH), 3.81 t (1H, H-3, *J* = 5, 4 Hz), 4.98 d.d (1H, H-8, *J* = 7, 3 Hz), 5.22 d.d (1H, H-8, *J* = 11, 3 Hz), 6.03 d.d (1H, H-7, *J* = 11, 7 Hz). ^{13}C NMR spectrum, δC, ppm: 25.2 (^{4}C), 25.9 (^{11}C), 26.0 (^{10}C), 27.3 (^{9}C), 29.7 (^{5}C), 82.52 (^{2}C), 82.8 (^{3}C), 83.1 (^{6}C), 111.6 (^{8}C), 144.3 (^{7}C).

The Second Compound:



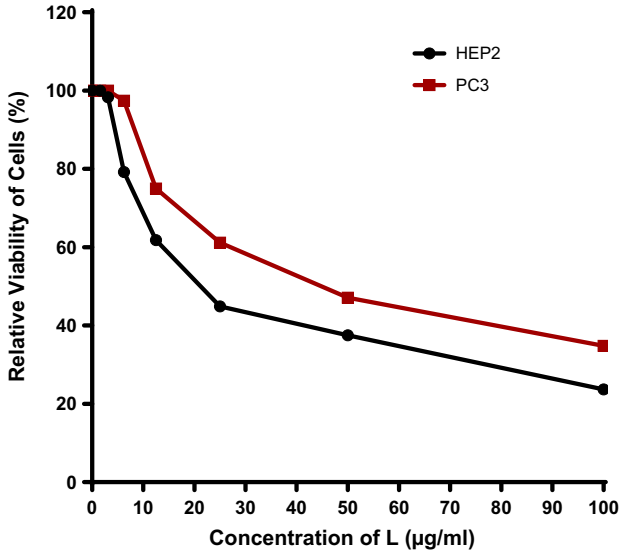


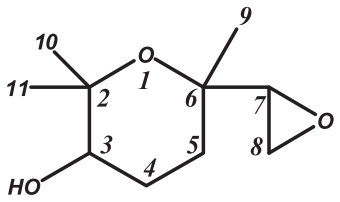
Fig. 2 Effect concentration of Linalool (I) on relative viability % epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) cell lines.

2(5-Methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol (IIa˝): ^{1}H NMR spectrum, δ, ppm: 1.20 s (3H, $^{6}CH_3$), 1.31 s (3H, $^{11}CH_3$), 1.33 s (3H, $^{10}CH_3$), 1.80 comp. pat. (2H, H-4), 1.98 comp. pat. (2H, H-3), 2.75 s (1H, OH), 3.88 t (1H, H-2, *J* = 5, 4 Hz), 4.98 d.d (1H, H-8 cis, *J* = 7, 3 Hz), 5.22 d.d (1H, H-8 trans, *J* = 11, 3 Hz), 5.88 d.d (1H, H-7, *J* = 11, 7 Hz). ^{13}C NMR spectrum, δC, ppm: 26.2(^{11}C), 26.5 (^{6}C), 26.6 (^{10}C), 26.8 (^{3}C), 37.5(^{4}C), 82.8 (^{9}C), 85.53 (^{5}C), 86.6 (^{2}C), 111.4 (^{8}C), 143.7 (^{7}C).

The mixture IIa/IIa′: Colorless oil, $C_{10}H_{18}O_2$ (*M* 170.250). IR spectrum, ν, cm^{-1}:

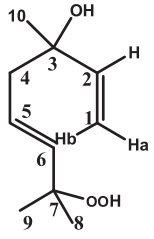
3439 (OH), 3087 (=CH), 2973 (CH str), 1642 (C=C), 1370 (C–O). GC–MS data: retention time 15.665 min; *m/z* (*I*rel, %): 170 (25) $(M)^+$, 152 (5) $(M\text{-}H_2O)^+$, 135 (20) $(M\text{-}CH_7O)^+$, 110 (10) $(C_7H_{10}O)^+$, 83 (50) $(C_6H_{11})^+$, 82 (10) $(C_6H_{10})^+$, 69 (1 0 0) $(C_5H_9)^+$.

Linalool Photoepoxidation:

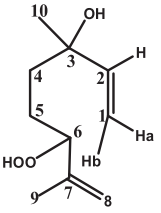


2,2,6-Trimethyl-6-oxiranyl-tetrahydro-pyran-3-ol (IIb): Colorless oil, $C_{10}H_{18}O_3$ (*M* 186.250). ^{1}H NMR spectrum, δ, ppm: 1.20 comp. pat. (2H, H-5), 1.24 s (6H, $^{10,11}CH_3$), 1.3 s (3H, $^9CH_3$), 1.89 comp. pat. (2H, H-4), 2.65 s (1H, OH), 2.74 d.d (1H, H-8, *J* = 18, 4 Hz), 2.88 d.d (1H, H^b-8, *J* = 18, 10 Hz), 3.01 d.d (1H, H-7, *J* = 10, 4 Hz), 3.86 d.d (1H, H-3, *J* = 7, 5 Hz). ^{13}C NMR spectrum, δC, ppm: 24.1 (^{9}C), 24.7 (^{4}C), 27.4 (^{10,11}C), 32.6 (^{5}C), 43.5 (^{8}C), 58.1 (^{7}C), 70.8 (^{3}C), 71.6 (^{6}C), 76.9 (^{2}C). GC–MS data: retention time 13.395 min; *m/z* (*I*rel, %): 186 (2) (*M*)+, 170 (15) $(M\text{-}O)^+$, 154 (10) $(M\text{-}O_2)^+$, 125 (20) $(M\text{-}C_2H_5O_2)^+$, 71(35) $(C_5H_{11})^+$, 55 (1 0 0) $(C_3H_3O)^+$, 43(90) $(C_3H_7)^+$.

Linalool Photooxygenation:



7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol(IIIa): Colorless oil, $C_{10}H_{18}O_3$ (*M* 186.250). ^{1}H NMR spectrum, δ, ppm: 1.3 s (3H, $^{10}CH_3$), 1.29 s (6H, $^{9,8}CH_3$), 2.25 d.d (1H, H^b –4, *J* = 13, 7 Hz), 2.33 d.d (1H, H^a –4, *J* = 13, 6 Hz), 5.63 d (1H, H-6, *J* = 16 Hz), 5.69 d.t(1H, H-5, *J* = 16, 7 Hz), 5.86 d.d (1H, H^{trans} –1, *J* = 16, 2.5 Hz), 5.89 d.d (1H, H^{cis} –1, *J* = 11, 2.5 Hz), 5.90 d.d (1H, H-2, *J* = 16, 11 Hz), 7. 7 s (1H, OOH). ^{13}C NMR spectrum, δC, ppm: 24.4(^{9}C), 24.4 (^{8}C), 31.9(^{10}C), 58.1 (^{4}C), 73.1(^{3}C), 81.8(^{7}C), 112.1 (^{1}C), 126.0(^{5}C), 138.1 (^{6}C), 143.8 (^{2}C). GC–MS data: retention time 16.125 min; *m/z* (*I*rel, %):186 (10) $(M)^+$, 170 (5) $(M\text{-}O)^+$, 153 (25) $(M\text{-}O_2H)^+$, 135(35) $(M\text{-}H_3O_3)^+$, 121 (8) $(M\text{-}CH_5O_3)^+$, 83 (70) $(C_6H_{11})^+$, 71(1 0 0) $(C_4H_7O_3)$, 43 (75) $(C_3H_7)^+$.



6-hydroperoxy-3,7-dimethylocta-1,7-dien-3-ol (IIIb): Colorless oil, $C_{10}H_{18}O_3$ (*M* 186.250). ^{1}H NMR spectrum, δ, ppm: 1.32 s (3H, $^{10}CH_3$), 1.72 s (3H, $^9CH_3$), 2.1–2.3 comp. pat. (4H, H-4,5), 4.30 comp. pat. (1H, H-6), 5.01 d.q (1H, H-8, *J* = 11, 1 Hz), 5.08 d.q (1H, H-8, *J* = 11, 1 Hz), 5.86 d.d (1H, H-1^{trans}, *J* = 16, 2.5 Hz), 5.89 d.d (1H, H-1^{cis}, *J* = 11, 2.5 Hz), 5.93 d.d (1H, H-2, *J* = 16, 11 Hz), 9.3 d (1H, OOH). ^{13}C NMR spectrum, δC, ppm: 17.4 (^{9}C), 25.01(^{5}C), 27.5 (^{10}C), 37.8 (^{4}C), 72.8 (^{3}C), 89.3 (^{6}C), 112.0 (^{1}C), 113.9 (^{8}C), 144.4 (^{7}C), 144.5 (^{2}C). GC–MS data: retention time 16.917 min; *m/z* (*I*rel, %): 186 (5) $(M)^+$, 170 (20) $(M\text{-}O)^+$, 153(16) $(M\text{-}O_2H)^+$, 135 (25) $(M\text{-}H_3O_3)^+$, 121(15) $(M\text{-}CH_5O_3)^+$, 83(50) $(C_6H_{11})^+$, 71(70) $(C_4H_7O_3)^+$, 43(1 0 0) $(C_3H_7)^+$.

3. Result and discussion

3.1. Chemistry

Linalool [3,7-dimethylocta-1,6-dien-3-ol] (**I**) is a alcoholic acyclic monoterpene, which is the component of the volatile oil of Ginger rhizome extracted from (***Zingiber officinale***).

The ^{1}H NMR spectrum of **I** contains three double doublets at δ 5.06, 5.21 & 5.91 ppm and a complex pattern at 5.13 ppm to the olefinic protons 1a, 1b, 2 & 6. Singlet signal at 2.19 ppm for hydroxyl proton.

Linalool was oxidized by Sc and Y-based bifunctional redox-acid catalysts to pyranoid and furanoid ethers (**IIa**/**IIa′**) (Perles et al., 2009; Dhakshinamoorthy et al., 2011) or by ethyl acetate, which forms peracetic acid as an intermediate in the presence of hydrogen peroxide with the aid of lipases (Patent Application, 2002).

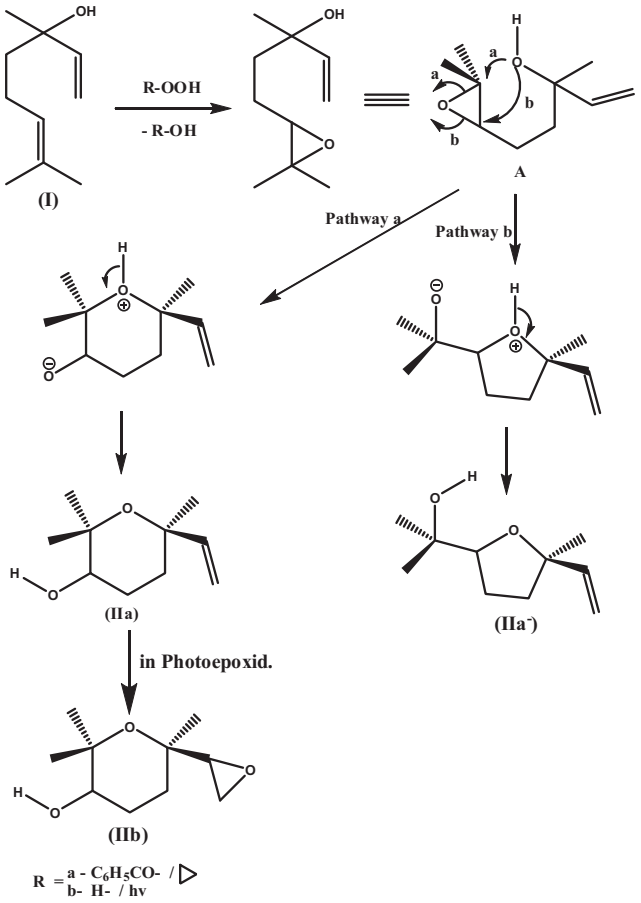
Photochemical epoxidation of linalool (**I**) with 30% hydrogen peroxide in ethanol using a sodium lamp gave a mixture of 2,2,6-trimethyl-6-vinyl-tetrahydro-pyran-3-ol **&** 2-(5-methyl-5-vinyl-tetrahydro-furan-2-yl)-propan-2-ol (**IIa**/**IIa′**) beside 2,2,8-trimethyl-6-oxianyl-tetrahydro-pyran-3-ol (**IIb**) at a ratio of 52:48 in an overall yield of about 40% (Scheme 1). Whereas, thermal epoxidation of linalool (**I**) with *m*-choroperbenzoic acid in chloroform at room temperature, obtained only a mixture of (**IIa**/**IIa′**), while no other products were detected (Scheme 1, Tables 1 and 2).

The structures of compounds **IIa**/**IIa′** and **IIb** were determined on the basis of spectral measurements. The ^{1}H NMR spectrum of **IIa**/**IIa′** showed one upfield triplet at δ 3.81 ppm for **IIa** and one upfield triplet at 3.88 ppm for **IIa′** due to methylene proton in position 3 and 2 respectively (6 for start **I),** the intensity ratio of the signals at δ 3.81and 3.88 ppm being 52:48 respectively**.** Isomers **IIa** and **IIa′** were characterized by same retention time, RT = 15.6665 min, and by the same *m/z* value (1 7 0) for the molecular ion (GC–MS data).

Compound **IIb** displayed in the ^{1}H NMR spectrum, three double doublet upfield at δ 2.74, 2.88 & 3.01 ppm from protons in 8a, 8b &7 in oxirane ring. The molecular ion of **IIb** had an *m/z* value of 186, with retention time RT = 13.395 min.

A probable mechanism for the formation of epoxy derivatives **IIa**, **IIa′**, and **IIb** is shown in Scheme 1.

Hydrogen peroxide or *m*-chloroperbenzoic acid attack the double bond at position 6, 7 in molecule **I** to give oxirane intermediate **A,** which decomposed and recyclized through pathway

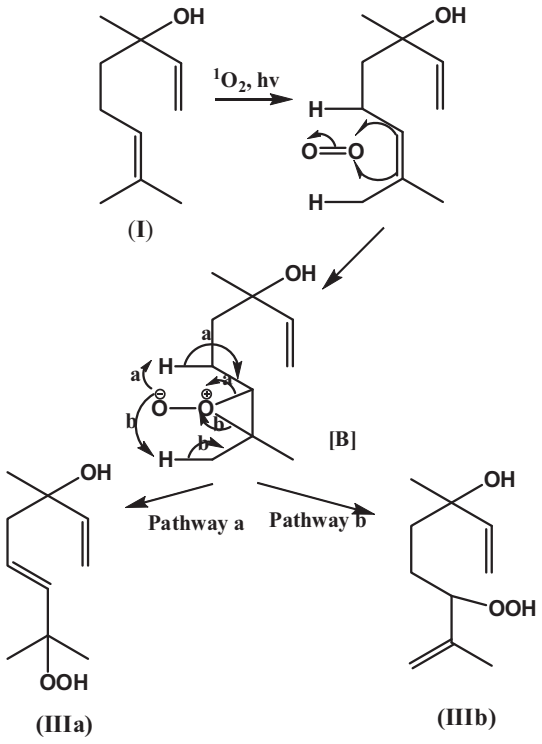


Scheme 1 Thermal and photochemical epoxidation of linalool (I).

a to form **IIa**, and pathway b to form **IIa′**. Alternatively, ability to interconverse is an essential property of ROS. For example, 1-electron reduction of H_2O_2 leads to the formation of $HO^{\bullet}$, indicating that formation of a less reactive form can promote the formation of more reactive forms (Mattila et al., 2015). Compound **IIb** can be produced when H_2O_2 molecule attack the double bond in **IIa** to form oxirane intermediate and elimination of water molecule.

Interestingly, the photooxygenation of compound **I** in the presence of tetraphenyl porphyrin (TPP) or chlorophyll (CP) as singlet oxygen sensitizer gave a mixture of 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol (**IIIa**) and 6-hydroperoxy-3,7-d imethylocta-1,7-dien-3-ol (**IIIb**) (Scheme 2) in an overall yield of 46 and 26.6%, respectively, the ratio **IIIa**: **IIIb** being 60:40 in all cases (according to the ^{1}H NMR data). The yield depended on the sensitizer activity and it decreased in going from TPP to CP in Table 3.

It has been succeeded in isolating between the mixture **IIIa**, **IIIb** and **IIIb** by column chromatography. The structure of the mixture **IIIa**, **IIIb** was confirmed by spectral data. Hydroperoxide **IIIa** displayed in the ^{1}H NMR spectrum a double multiplet at δ 5.69 ppm and a singlet at 7.7 ppm from the olefinic proton in positions 5 and OOH proton, respectively. ^{1}H NMR spectrum of **IIIb** contained two double triplets at δ 5.01 & 5.08 ppm and a doublet at 9.3 ppm to the olefinic protons 8a, 8b, and OOH proton respectively. Compounds **IIIa**

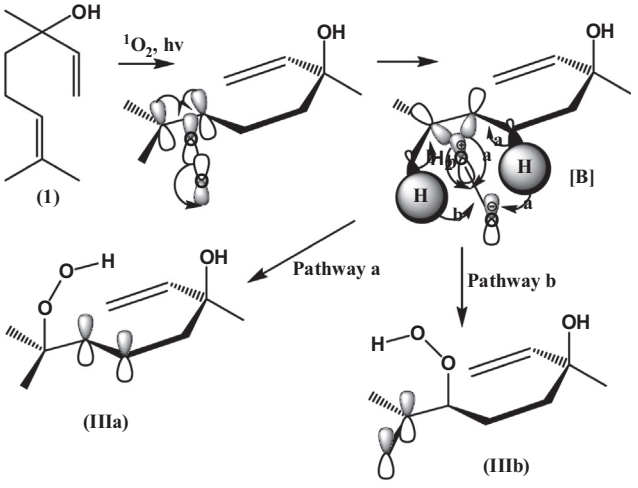


Scheme 2 Photosensitized oxidation of linalool (I).

and **IIIb** showed in the mass spectra the molecular ion peak with m/z 186. Compounds **IIIa** and **IIIb** were characterized by a retention time of (GC–MS), 16.649 & 16.125 min respectively. The mixture IIIa and IIIb, cannot be separated, due to their very near retention time.

In the photosensitized oxidation of linalool (**I**), hydroperoxides **IIIa** and **IIIb** are likely to be formed through peroxirane intermediate **C** which is stabilized along two possible pathways *a* and *b* (Elgendy, 1998) (Scheme 2 and 3).

On the other hand, linalool will be oxidized when exposed to air at normal temperature and pressure. The hydroperoxides **IIIa** and **IIIb** were identified, via GC–MS as the main oxidation products (Backtorp, 2006).



Scheme 3 Photosensitized oxidation of linalool (I).

3.2. Biological evaluation

3.2.1. DNA damage activity of new products

Epe et al. (1993) reported that some hydroperoxides can be damaged DNA photochemically. Therefore, a mixture of **IIIa** and **IIIb** was examined for DNA-damaging activity. A solution of hydroperoxide **IIIa** and **IIIb** in ethanol was mixed with DNA in saline and irradiated using a sodium lamp. It has been proved that (see Experimental) it caused moderate and high degrees of DNA degradation, when the irradiation time was prolonged to 45 h.

Allylic hydroperoxides can be formed from the autoxidation of terpenes which presence in fragrance and may be caused an allergic action when it contact the skin (Kao et al., 2011). Allylic hydroperoxides are binding covalently to proteins in the skin through radical pathways. 7-Hydroper oxy-3,7-dimethylocta-1,5-dien-3-ol **(IIIa)** is responsible for the sensitizing potential acquired by autoxidized linalool. Adducts, are formed between the hydroperoxides and N-acetyl histidine methyl ester, via radical mechanisms (Kao et al., 2014).

3.2.2. Antioxidant activity of linalool (*I*) in vitro

It well known that, free radicals play a main role in many chronic diseases such as cancer and heart diseases. They are involved in the lipid per-oxidation reactions (Halliwell and Gutteridge, 2000). Thence, the potency to quench free radicals is an antioxidant feature to reduce cell damage. In numerous researches *in vitro*, the mono terpene appeared expected antioxidant activity (Elgendy, 2004).

3.2.2.1. Choice of the method for determination of antioxidant activities. Frenkel et al. (1994) and Koleva et al. (2002) can be measured antioxidant activity using different methods. Erythrocyte hemolysis, RANCIMAT and ABTS can be applied as easy technique to estimate the antioxidant activity of mono terpenes (Tomaino, 2005; El-Gazzar et al., 2009).

Though the RANCIMAT test is usually used in governmental analytical laboratories and the food industry (Pajero et al., 2003; Frenkel, 1993).

RANCIMAT test is not appropriate for such examination, because mono terpene sample can be evaporated, previously and delay measurements.

3.2.2.2. Anti-oxidant activity screening assay ABTS. In this study, linalool (I) was tested for antioxidant activity which appeared medium ability to prohibit lipid per-oxidation. The powerful efficiency is declared by a higher antioxidant value measured by ABTS assay. It has been suggested that linalool (I), could be applied as an expected source of natural antioxidants with wide enforcement in food manufactures. The result is summarized in Table 4.

3.2.2.3. Anti-oxidant activity screening assay erythrocyte hemolysis. Linalool (I) was examined for anti-oxidant activity as reflected in the ability to inhibit lipid peroxidation rate erythrocyte hemolysis. It manifested potent anti-oxidative activity in the lipid peroxidation assay which showed high inhibitory activity in the hemolysis assay. The results indicated that it may have some protective activity in Table 5.

3.2.3. Cytotoxicity assay through hepatoprotective activity evaluation in vitro:

Some of mono terpenes have anti-carcinogenic activity and they can be used on a long-term, without any genotoxic risk (Hassan et al., 2010).

The cytotoxic activity of linalool (I) was tested on epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) immortal cell line, which used in laboratory study and the most common utilized human cell lines (Bladt et al., 2013; Mashhadian et al., 2009). It submitted good activity on the cell lines. It showed more inhibition rate. The IC50 values against epdermoid carcinoma (HEP2) and Human prostate cancer (PC-3) proved that the cytotoxicity of linalool (I) decreased when its concentricity increase. Otherwise, the IC50 values versus epdermoid carcinoma (HEP2), it has medium effect. Whereas, in case of human prostate cancer (PC-3) indicated that it has weak effect, in Tables 6 and 7 and Figs. 1 and 2.

4. Conclusion

We can be concluded that linalool, which extracted from ginger has good antioxidant potential. Linalool was evaluated for anti-oxidant, using erythrocyte hemolysis and ABTS methods. It showed medium inhibitory effect in case of erythrocyte hemolysis and low inhibitory effect in case of ABTS method. In addition, the cytotoxic activity of linalool (**I**) was tested against epdermoid carcinoma (HEP2), it has medium effect. Whereas, in case of human prostate cancer (PC-3), it has weak effect. On the other hand, this spice can be used to produce novel natural antioxidants as well as flavoring agents that can be used in different food products. Novel hydroperoxides derived from Linalool can be obtained by photosensitized oxygenation, it showed high degrees of DNA degradation, when the irradiation time was prolonged to 45 h. Probably, such hydroperoxides are generated *in situ* upon irradiation of linalool in the presence of DNA, and they can be used as anticancer drug. Therefore, it appears to be relevant to clarify biological outcome of hydroperoxides with DNA and many cell constituents. The genotoxicity of such DNA intercalators having a more oxidative possibility was not examined formerly.

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References

Altman, R.D., Marcussen, K.C., 2001. Effects of a ginger extract on knee pain in patients with osteoarthritis. Arthritis Rheum. 44, 2531–2538.

Backtorp, C., Johnson Wass, J.R.T., Panas, I., Skold, M., Borje, A., Nyman, G., 2006. Theoretical investigation of linalool oxidation. J. Phys. Chem. A 110 (44), 12204–12212.

Bentley, R., 2006. Enantiomer discrimination. Chem. Rev. 106, 4099–4112.

Bladt, T.Th., Frisvad, J.C., Knudsen, P.B., Larsen, Th.O., 2013. Anticancer and antifungal compounds from aspergillus, penicillium and other filamentous fungi. Molecules 18, 11338–11376.

Choudhury, D., Das, A., Bhattacharya, A., Chakrabarti, G., 2010. Aqueous extract of ginger shows antiproliferative activity through disruption of microtubule network of cancer cells. Food Chem. Toxicol. 48 (10), 2872–2880.

Cho, S.H., Ma, B.Q., Nguyen, S.T., Hupp, J.T., Albrecht-Schmitt, T. E., 2006. A metal-organic framework material that functions as an enantioselective catalyst for olefin epoxidation. Chem. Commun., 2563–2565

Cho, S.H., Gadzikwa, T., Afshari, M., Nguyen, S.T., Hupp, J.T., 2007. [Bis(catechol)salen]Mn III Coordination Polymers as Support-Free Heterogeneous Asymmetric Catalysts for Epoxidation. Eur. J. Inorg. Chem. 31, 4863–4867.

Corma, A., Dominguez, I., Domenech, A., Fornes, V., Gomez-Garcia, C.J., Rodenas, T., Sabater, M.J., 2009. Enantioselective epoxidation of olefins with molecular oxygen catalyzed by gold(III): A dual pathway for oxygen transfer. J. Catal. 265 (2), 238–244.

Dhakshinamoorthy, A., Alvaro, M., Corma, A., Garcia, H., 2011. Delineating similarities and dissimilarities in the use of metal organic frameworks and zeolites as heterogeneous catalysts for organic reactions. Dalton Trans. 40, 6344–6360.

El-Gazzar, A.B.A., Youssef, M.M., Youssef, A.M.S., Abu-Hashem, A.A., Badria, F.A., 2009. Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti- oxidant, anti-inflammatory and analgesic activities. Eur. J. Med. Chem. 44, 609–624.

Elgendy, E.M., 1998. Photooxygenation of natural limonene. Chin. Pharm. J. 50, 225.

Elgendy, E.M., Abou-Elzahab, M.M., 1998. Synthesis of Imperatorin Bromohydroperoxide derivatives. Alex. J. Pharm. Sci. 12, 21–24.

Elgendy, E.M., 2004. Photooxygenation of Natural γ-Terpinene. Boll. Chim. Farmac. – Anno 143, 337–339.

Elgendy, E.M., Al-Ghamdy, H., 2007. Thermal and Photooxidation reactions of the steroids: β-sitosterol, stigmasterol and diosgenin. Taiw. Pharm. J. 59, 113–132.

Elgendy, E.M., 2008. The potential biologically active epoxide, hydroperoxides and endoperoxide derivatives derived from natural monoterpene β-myrecene. Aust. J. Bas. Appl. Sci. 2 (2), 221–224.

El-ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S.H., Nadeem, M.A., 2010. Comparative study on chemical composition and antioxidant activity of ginger (Zingiber officinale) and Cumin (Cuminum cyminum). J. Agric. Food Chem. 58, 8231–8237.

Epe, B., Häring, M., Ramaiah, D., Stopper, H., Abou- Elzahab, M. M., Adam, W., Saha-Möller, C.R., 1993. DNA damage induced by furocoumarin hydroperoxides plus UV (360 nm). Carcinogenesis 14 (11), 2271–2276.

Frenkel, E.N., 1993. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends Food Sci. Technol. 4, 220–225.

Frenkel, E.N., Huang, S.W., Kanner, J., German, J.B., 1994. Interfacial phenomena in the evaluation of antioxidants: bulk oils vs emulsions. J. Agric. Food Chem. 42, 1054–1059.

Geraghty, N.W.A., 2005. Photochemistry. Royal Soc. Chem., London, p. 116.

Grant, K.L., Lutz, R.B., 2000. Alternative therapies: ginger. Am. J. Health-Syst. Pharm. 57, 945–947.

Halliwell, B., Gutteridge, J.M.C., 2000. Free Radicals in Biology and Medicine. Oxford University Press, Oxford.

Harold, M.-G., 2004. On Food and Cooking: The Science and Lore of the Kitchen. Scribner, New York, pp. 425–426.

Hassan, S.B., Muhtasib, H.G., Kultima, H.G., Larsson, R., 2010. Alpha terpineol: A potential anticancer agent which acts through suppressing NF-κB signaling. Anticancer Res. 30 (6), 1911–1919.

Kao, D., Chaintreau, A., Lepoittevin, J.P., Giménez-Arnau, E., 2011. Synthesis of allylic hydroperoxides and EPR spin-trapping studies on the formation of radicals in iron systems as potential initiators of the sensitizing pathway. J. Org. Chem. 76 (15), 6188–6200.

Kao, D., Chaintreau, A., Lepoittevin, J.-P., Giménez-Arnau, E., 2014. Mechanistic studies on the reactivity of sensitizing allylic hydroperoxides: investigation of the covalent modification of amino acids by carbon-radical intermediates. Toxicol. Res. 3, 278–289.

Kochevar, I.E., Dumn, D.A., 1990. Photochemistry and the nucleic acids. In: Morrison, H. (Ed.), Bioorganic Photochemistry. Wiley, New York, p. 273.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13, 8–17.

Lissi, E., Modak, B., Torres, R., Escobar, J., Urzua, A., 1999. Total antioxidant potential of resinous exudates from Heliotropium species, and a comparison of ABTS and DPPH methods. Free Radic. Res. 30, 471e7.

Mashhadian, N.V., Emami, S.A., Oghazian, M.B., Vosough, R., 2009. The cytotoxicity evaluation of seven species of Artemisia on human tumor cell lines. Pharmacologyonline 1, 229–242.

Mattila, H., Khorobrykh, S., Havurinne, V., Tyystjärvi, E., 2015. Reactive oxygen species: reactions and detection from photosynthetic tissues. J. Photochem. Photobiol., B: Biol. 152, 176–214.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Pajero, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Saavedra, G., Murcia, M.A., Jimenez, A.M., Codina, C., 2003. Investigation of bolivian plant extracts for their radical scavenging activity and antioxidant activity. Life Scien. 73, 1667–1681.

Patent Application, 2002. Process For The Preparation Of Linalool Oxide Or Of Linalool Oxide-containing Mixtures. US 2002/0086376 A1.

Perles, J., Snejko, N., Iglesias, M., Monge, M.A., 2009. 3D scandium and yttrium arenedisulfonate MOF materials as highly thermally stable bifunctional heterogeneous catalysts. J. Mater. Chem. 19, 6504–6511.

Richter, S., Gatto, B., Fabris, D., Takao, K.-I., Kobayashi, S., Palumbo, S.M., 2003. Clerocidin alkylates DNA through its epoxide function: evidence for a fine tuned mechanism of action. Nucleic Acids Res. 31 (17), 5149.

Sköld, M., Börje, A., Harambasic, E., Karlberg, A.-T., 2004. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. Chem. Res. Toxicol. 17, 1697–1705.

Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., Saija, A., 2005. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem. 89, 549–554.

Yarovaya, O.I., Korchagina, D.V., Gatilov, Yu.V., Barkhash, V.A., 2002. Acid-catalyzed reactions of camphene and α-fenchene epoxides. Russ. J. Org. Chem. 38 (6), 810.

Yarovaya, O.I., Korchagina, D.V., Salomatina, O.V., Polovinka, M. P., Barkhash, V.A., 2003. Synthesis of heterocyclic compounds in acid-catalyzed reactions of citral epoxides. Mendeleev Commun. 13 (1), 27.